with SEQ ID NO: 7;

to obtain an amplified sample;

(d) determining the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample;

A3 CONT

(e) comparing the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample with a predetermined threshold level for *DPD* expression;

(f) determining a 5-Fluorouracil-based chemotherapeutic regimen for said patient based on the difference in amount of *DPD* mRNA in the amplified sample and the threshold level for *DPD* gene expression.

24. (Amended) The method of claim 23, wherein said predetermined threshold level of *DPD* gene expression is about 2.0 to about 2.5 times that of an internal control gene expression level.

25. The method of claim 23, or 24, wherein said internal control gene is β-actin.

REMARKS

Claims 6-11, 17-22, and 26 have been canceled. Claims 12, 13, 23 and 24 have been amended. No new matter has been added by these claim amendments. Claims 14-16 and 25 remained unchanged. Accordingly, claims 12-16 and 23-25 are pending in this application.

Double Patenting

Applicant has canceled Claims 6-11, 17-22, and 26, which subject matter is being pursued in a related application. Accordingly, the provisional double patenting rejection is rendered moot. Applicants respectfully request withdrawal of this ground of rejection

Rejection under 35 U.S.C. § 112, First Paragraph - Enablement

The Examiner rejects claims 6-26 under 35 U.S.C. § 112, first paragraph, for allegedly

encompassing subject matter that is not sufficiently enabled by the specification. The Examiner alleges that although the specification is enabling for some internal controls, the specification does not provide sufficient enablement for *β-actin* as an internal control. Applicant respectfully disagrees with the Examiner's rejection.

The pending claims recite a method for determining a 5-Fluorouracil-based chemotherapeutic regimen for treating a tumor in patient involving obtaining a tumor sample from the patient; isolating mRNA from said tumor sample; subjecting the mRNA to amplification using a pair of oligonucleotide primers to obtain an amplified sample, determining the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample; comparing the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample with a predetermined threshold level for *DPD* expression; determining a 5-Fluorouracil-based chemotherapeutic regimen for said patient based on the difference in amount of *DPD* mRNA in the amplified sample and the threshold level for *DPD* gene expression. Further, dependent claims are directed to the above method where the predetermined threshold level of *DPD* gene expression is about 2.0 to about 2.5 times that of an internal control gene expression level.

The Examiner argues that *β-actin* is not a suitable internal control gene in tumor cells because it is not possible to determine the effects of a patient's individual treatment history on the gene expression of *β-actin* or other cellular markers. To support his position, the Examiner cites Willhauck *et al.*, BioTechniques 25:656-659 (October 1998). The Examiner quotes Willhauck *et al.* which states, "the increasing number of *GAPDH* and *β-actin* pseudogenes which can be amplified even if mRNA-specific primers were designed, can lead to an overestimation of the RT efficacy." Willhauck *et al.* teaches spiking a blood sample being analyzed for *tyrosinase* gene expression by melanoma cells, with a pre-set number of Jurkat T-cells to act as an internal control.

Additionally, the Examiner cites Selvey *et al.*, Molecular and Cellular Probes, 15: 307-311, (2001). The Examiner quotes Selvey *et al.* as stating: "These results clearly demonstrate

the unsuitability of *β-actin* as an internal control for gene expression studies." However, Selvey *et al.* only teaches that the use of *β-actin* is not appropriate as an internal control gene in a "specific case" (page 308, left column) i.e., when assaying the relative expression levels of *MT1-MMP membrane type1 - matrix metalloproteinase (MT1-MMP)* in fibroblasts grown on a coat of matrigel matrix. Selvey *et al.* also states that although there is evidence that *β-actin* may not be appropriate as an internal control gene in all circumstances, it nonetheless is the, "most widely used internal control in molecular biology," and that it, "remains a popular choice for countless RT-PCR applications." (p. 308, left column). In view of their observations with respect to *β-actin* as an internal control gene when assaying the relative expression levels of *MT1-MMP* in fibroblasts grown on a coat of matrigel matrix, Selvey *et al.* suggest that investigators using this gene as an internal control should first, "demonstrate to a satisfactory degree, that it is not regulated in their specific application." (p. 310, right column).

Applicant respectfully submits that regardless of the Examiner-cited references, one of skill in the art would be more than satisfied that *β-actin* is appropriate as an internal control gene when assaying the relative expression level of *DPD* and other genes in various tissues.

For instance, Salonga *et al.*, Clinical Cancer Research, 6:1322-1327, (April 2000) reported that when using *β-actin* as an internal control, "a linearity is obtained between gene expression values determined by RT-PCR and protein content determined by immunohistochemistry." (p. 1323, right column). As such, they reported relative expression levels of *Thymidylate Synthase (TS)* and *DPD* as a ratio of *TS* and *DPD* RT-PCR products to *β-actin* RT-PCR products, respectively, in 33 colorectal tumors. Johnston, *et al.*, Cancer Research, 55(7):1407-12, (April 1995), reported a "close linear relationship between TS protein expression and *TS* gene expression" when using *β-actin* as an internal control gene in 21 tumor samples (9 colorectal and 12 gastric, p. 1409, right column). For illustrative purposes, Johnston and Salonga teach one of ordinary skill in the art that if *TS* gene expression is reported to be 2 times *β-actin* expression in a sample A and 3 times *β-actin* expression in another sample B, that the immunohistochemical TS protein signal in sample B was consistently observed to be 50% greater

than in sample A. The consistency of this observation was characterized as a "close linear relationship" and teaches that *β-actin* expression provides a reliable control.

More recently, Gustavsson *et al.*, ASCO Annual Meeting of 2002, Abstract 457, reported measuring relative *TS, DPD, TP*, folypolyglutamate synthetase (*FPGS*) and gamma-glutamyl hydrolase (*GGH*) mRNA expression levels in paraffin-embedded primary tumor tissues from 26 patients with colorectal cancer using *β-actin* as an internal control gene.

(http://www.asco.org/asco/ascoMainConstructor/1,1003,_28-003-00_12-002326-00_29-00A-00_18-002002-00_19-00457,00.asp).

Additionally, Eads *et al.*, Cancer Research, 59:2302-2306 (May 15, 1999) teach the measurement of several DNA Methyltransferase (*DNMT1, DNMT3A, DNMT3B*) genes' expression relative to *β-actin*, *RNA polymerase II large subunit* as well as *histone H4* and *PCNA* gene expression in 25 human colorectal adenocarcinoma tumor tissue samples and 25 matching non-tumor mucosal samples. The study determined that *β-actin* and *RNA polymerase II large subunit* genes, considered to be non-proliferation associated genes, were appropriate internal control genes, whereas the proliferation associated genes for *histone H4* and *PCNA* were not. (p. 2304, paragraph bridging right and left columns).

Finally, to further cement the fact that one of ordinary skill in the art would readily accept the suitability of *β-actin* as an internal control gene, Applicant herewith provides a list of dozens of the present inventor's own peer-reviewed studies reporting the successful use of *β-actin* in a large variety of gene expression and tissue applications. Please see Appendix A.

Moreover, other groups have reported similarly satisfactory results using *GAPDH* as an internal control gene, when analyzing *DPD* expression in various tissues. Takechi *et al.*, Japanese Journal of Cancer Research, 89: 1144-1153 (November 1998) report the relationship between *DPD* protein levels and *DPD* gene expression relative to *GAPDH* in pancreatic carcinoma and fibrosarcoma cells grown in culture and in nude mice. Similarly, Uetake *et al.*, Clinical Cancer Research, 5:2836-2839 (October 1999), report the relationship between intratumoral *DPD* protein activity and *DPD* gene expression relative to *GAPDH* in human colorectal tumor samples and paired non-tumoral tissue samples. Uetake *et al.* state that an

internal control gene, such as *GAPDH*, "is expressed in a constant per-cell level," and is useful for normalization of target gene expression. (p. 2837, left column). Finally, Johnson *et al.*, Analytical Biochemistry 278: 175-184 (2000), assayed *DPD* gene expression relative to *GAPDH* in colorectal and liver tumors by Real-Time RT-PCR using a TaqMan® system similar to that exemplified in the subject application. The investigators found that as opposed to the semi-quantitative RT-PCR of the past, the Real-Time RT-PCR methodology utilizing *GAPDH* as an internal control gene is "accurate and reproducible" and has, "low inter- and intra-assay variations." (p. 183, right column). Moreover, they suggest that it might someday even be possible to, "quantitate *DPD* mRNA in archival material such as paraffin-embedded tissues." (p. 183, left column). The authors point out that this would be advantageous because, "the response to chemotherapy is known," in the archival samples, "correlations between response to 5-FU and *DPD* mRNA levels could readily be determined." (p. 183, left column). The subject application provides a road map for carrying out such studies by providing a method for determining relative *DPD* expression in a tissue.

In view of these extensive findings, Applicant respectfully asserts that one of ordinary skill in the art would readily accept the utility of *β-actin* and *GAPDH* as suitable internal control genes for determining the relative *Dihydropyrimidine dehydrogenase* (*DPD*) gene expression in a tissue sample. Again, even if Selvey *et al.* state that there is evidence that *β-actin* may not be appropriate as an internal control gene in all circumstances, it nonetheless is the, "most widely used internal control in molecular biology," and that it, "remains a popular choice for countless RT-PCR applications." As such, one of skill in the art would know how to make and use the claimed invention without undue experimentation with respect to the claim recitations "internal control gene" and "*β-actin*" as an internal control gene.

Setting aside these peer-reviewed studies, even if one of skill in the art were not to consider *GAPDH* or *β-actin* to be appropriate internal controls genes for purposes of the claims at issue, one of skill in the art would readily and without undue experimentation, chose any other internal control gene they deem appropriate to make and use the claimed invention.

The specification defines internal control genes as including any constitutively or globally expressed gene whose presence enables an assessment of relative *DPD* mRNA levels. The specification further indicates that such internal control genes allow for a determination of the overall constitutive level of gene transcription and control for variations in RNA recovery. As such, one of skill in the art would instantly recognize that any gene satisfying these criteria would be appropriate as an internal control gene. As examples the specification lists the cyclophilin gene, *β-actin* gene, the transferrin receptor gene, *GAPDH* gene, and the like.

Regardless of whether or not a small handful of studies suggest, that *β-actin* and *GAPDH* might be suitable internal control genes for assaying *DPD* expression in tissue samples, the skilled artisan could readily pick any genes such as *cyclophilin, transferrin receptor, 18S RNA, Peptidylprolyl isomerase A (PPIA),* or any other well known internal control gene.

The Examiner even indicates that the specification is enabling for some internal controls. Unless a particular internal control gene is shown to be clearly inappropriate for detecting a specific gene in from a particular cell-type in a specific context, e.g. when assaying the relative expression levels of MT1-MMP in fibroblasts grown on a coat of matrigel matrix, then for purposes of the claimed methods for determining the relative level of *Dihydropyrimidine Dehydrogenase* (*DPD*) gene expression in a tissue sample, the identity of the internal control gene is irrelevant. As such, because the specification provides enablement for some internal control genes, as indicated by the Examiner, then the Applicant is entitled any internal control that one of skill in the art would consider suitable for a method of determining the relative level of *Dihydropyrimidine Dehydrogenase* (*DPD*) gene expression in a tissue sample. Such a decision requires no undue experimentation given the fact that there are many commercial RT-PCR kits available that supply a reagents for number of internal control genes. Accordingly, Applicant respectfully requests withdrawal of this ground for rejection.

Rejection under 35 U.S.C. § 112, First Paragraph - Written Description

The Examiner has rejected claims 6-26 under 35 U.S.C. § 112, first paragraph, as

allegedly encompassing subject matter lacking sufficient written description. The Examiner alleges that the specification discloses SEQ ID Nos: 1, 2, 7 and 8, and no specific examples of nucleic acids that are "substantially identical" to them. The Examiner further argues that "substantially identical" encompasses a genus of oligonucleotides that are not described in the specification and as a result one of ordinary skill in the art would not be convinced that the Applicant was in possession of the claimed genus at the time of filing.

The claims have been amended to remove the "substantially identical" language. The claims have been amended to add three additional limitations to the claimed primers: 1) an "at least 80% identical" requirement; 2) a well defined functional requirement, i.e. that the oligo primers are capable of amplifying a particular portion of a particular Exon of *DPD* mRNA (either Exon 1 or 6, depending on the claim), and the *DPD* mRNA is from FPE tissue; and 3) a stringent hybridization requirement. Applicant submits that amended claims now render this ground of rejection moot. However, in so much as the Examiner feels that the rejection applies to the amended claims, applicant respectfully address this rejection as follows.

The Examiner refers to *The Regents of the University of California v. Eli Lilly and Co.,* 43 U.S.P.Q.2d 1398 (Fed. Cir.1997)(hereinafter referred to as *UC v. Eli Lilly*) as a basis for the Written Description rejection. *UC v. Eli Lilly* states that a claim containing only functional limitations without structural characteristics is insufficient to satisfy the Written Description requirement. The situation in *UC v. Eli Lilly* is not present in the subject application, since the present claims have been amended to include two structural characteristics (i.e. percent identity and stringent hybridization requirements, as well as a functional limitation (i.e. capable of amplifying a specified portion of *DPD* mRNA isolated from fixed and paraffin embedded (FPE) tissue).

Further, the United States Court of Appeals for the Federal Circuit, in *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 296 F.3d 1316 (Fed. Cir. 2002)(hereinafter referred to as *Enzo*), has further clarified the Written Description requirement and provided more guidance. In characterizing its earlier case of *UC v. Eli Lilly*, the Court in *Enzo* noted that the written description was not met as

"the specification and generic claims to all cDNAs encoding for vertebrate or mammalian insulin did not describe the claimed genus because they did not set forth any common features possessed by members of the genus that distinguished them from others." *Enzo*, 296 F.3d at 1327. Unlike the case in *UC v. Eli Lilly*, in the present application, the claims set forth common features possessed by members of the genus that distinguishes them from others. The claims set forth the common feature that all of the claimed oligonucleotide primers must be capable of performing a very specifically defined function, in addition to having at least 80% and hybridize under stringent conditions (which is defined in the specification as what one in the art would consider "highly stringent conditions"). Thus, applicants respectfully submit that the shortcomings found in the claims at issue in *UC v. Eli Lilly*, are not present in the subject claims.

Applicants respectfully also submit that the Written Description Guidelines provide guidance of the Written Description requirements. Example 9 of the Written Description Guidelines (Federal Register, Vol. 66, No. 4, Friday, January 5, 2001), states that:

> [A] person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because highly stringent conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, **since highly stringent hybridization conditions *in combination* with the coding function of DNA** and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

(emphasis added).

Thus, it appears that the Guidelines reveal that the written description requirement would be met by claims reciting high stringent hybridization conditions in combination with a functional requirement. In addition, to discussing *UC v. Eli Lilly*, the Court in *Enzo* noted that the PTO "has determined that such claims may be adequately described if they hybridize under highly stringent conditions to known sequences because such conditions dictate that all species within the genus will be structurally similar," citing Example 9 of the Guidelines at 35-37. *Enzo*, 296 F.3d at 1327. Further, the Court stated that "it is not correct, however, that all functional descriptions of genetic material fail to meet the written description requirement." *Enzo*, 296 F.3d

at 1324. The Court further quoted the Guidelines referenced above: "the written description requirement can be met by 'showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics. . .i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.'" The Federal Circuit thereafter stated that it is "persuaded by the Guidelines" and "adopts the PTO's standard for determining compliance with the written description requirement." *Enzo*, 296 F.3d at 1325.

As such, the Applicant respectfully submits that the amended claims at issue meet the written description requirement as they recite both a functional requirement (capable of amplifying a specified portion of *DPD* mRNA from FPE tissue) and several structural requirements (stringent hybridization conditions as clearly defined in the specification and understood to be high stringent conditions by one skilled in the art) and a percent identity requirement.

For further guidance, the Federal Circuit stated in *Enzo* that the specification and claims at issue in *Enzo* would meet the written description requirement "if the **functional characteristic** of preferential binding to N. gonorrhoeae over N. meningitidis were coupled with a disclosed **correlation between that function and a structure** that is sufficiently known or disclosed." (emphasis added). *Enzo*, 296 F.3d at 1324-25. The claims of the subject application recite functional characteristic (capable of amplifying certain exons of *DPD* mRNA from FPE tissue) coupled to a structure that is sufficiently known or disclosed (at least 80% identity and hybridizes under stringent conditions to a complement of the enumerated SEQ ID NO.). Accordingly, Applicant submits that the application and the claims satisfy the 112, first paragraph written description requirement. Withdrawal of this rejection is respectfully requested.

Rejection under 35 U.S.C. § 103(a)

The Examiner rejects claims 6-13, 15-24, and 26 under 35 U.S.C. § 103 (a) as allegedly

being unpatentably obvious over Gonzales *et al.*, U.S. Patent No. 6,015,673 in view of Willhauck *et al.*, BioTechniques, 1998, 25:655-659. The Examiner opines that SEQ ID NO: 5 of Gonzales *et al.* teaches an oligonucleotide with 14 of 18 nucleotides identical to claimed SEQ ID NO: 1. The Examiner further alleges that such an oligonucleotide is 77% identical with claimed SEQ ID NO: 1. Applicants respectfully point out that SEQ ID NO:1 has 19 and not 18 bases and thus Gonzales only has 14 of 19 nucleotides identical with SEQ ID NO:1 for a 73% identity.

Claim 12 has been amended to recite that the oligonucleotide is at least 80% identical to SEQ ID. NO: 1. Accordingly, applicants submit that this ground of rejection is moot as Gonzales' oligonucleotide is not at least 80% identical to SEQ ID No:1. Further, there is no teaching nor suggestion that the Gonzales nucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2.

Although, on its face, it appears that Gonzales *et al.'s* RTF1 and RTR1 may be useful for amplifying a 1.5kb *DPD* cDNA fragment, there is nothing in Gonzales *et al.* that would suggest to one of skill in the art that either RTR1 nor the allegedly anticipatory RTF1, either together or individually, would be appropriate for amplifying mRNA isolated from fixed and paraffin embedded tissue. Therefore, one of skill in the art would not be motivated to modify either RTF1 or RTR1, let alone search for or design a purified oligonucleotide consisting of the sequence of SEQ ID NO: 1, or a sequence which is at least 80% identical therewith and hybridizes to a complement of SEQ ID NO: 1 under stringent conditions; wherein the isolated and purified oligonucleotide is capable of amplifying a portion of a *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2, as claimed. Accordingly, it can further be concluded that Gonzales *et al.* cannot be asserted to suggest or contemplate the claimed oligonucleotides either.

Moreover, even if one of skill in the art was motivated to draw on the teachings of Willhauck *et al.*, the combination of these references does not render the claims unpatentably obvious because, even together, they do not teach, suggest nor contemplate an oligonucleotide

consisting of the sequence of SEQ ID NO: 1, or a sequence which is at least 80% identical therewith and hybridizes to SEQ ID NO: 1 under stringent conditions; wherein the isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2.

In view of the remarks and amendments made herein, Applicant respectfully asserts that the rejection is traversed, and withdrawal thereof is respectfully requested.

CONCLUSION

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 11-0600.

Respectfully submitted,

KENYON & KENYON

Date: 1/24/03

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MARKED UP VERSION TO SHOW CHANGES MADE

12. (Amended) A method for determining a 5-Fluorouracil-based chemotherapeutic regimen for treating a tumor in patient comprising:

(a) obtaining a tumor sample from the patient;

(b) isolating mRNA from said tumor sample;

(c) subjecting the mRNA to amplification using a pair of oligonucleotide primers having the sequence of SEQ ID: 1, [or which is substantially identical thereto] or an oligonucleotide primer at least 80% identical therewith and hybridizes to a complement of SEQ ID NO: 1 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2, and

an oligonucleotide having the sequence SEQ ID: 2, [, or which is substantially identical thereto] or an oligonucleotide primer at least 80% identical therewith and hybridizes to a complement of SEQ ID NO: 2 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 1;

to obtain an amplified sample,

(d) determining the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample;

(e) comparing the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample with a predetermined threshold level for *DPD* expression;

(f) determining a 5-Fluorouracil-based chemotherapeutic regimen for said patient

based on the difference in amount of *DPD* mRNA in the amplified sample and the threshold level for *DPD* gene expression.

13. (Amended) The method of claim 12, wherein said predetermined threshold level of *DPD* gene expression is about 2.0 to about 2.5 times that of an internal control gene expression level.

14. The method of claim 12 or 13, wherein said internal control gene is β-actin.

15. The method of claim 13, wherein the tumor sample is fixed and embedded after being obtained.

16. The method of claim 13, wherein the mRNA is isolated in the presence of an effective amount of chaotropic agent.

23. (Amended) A method for determining a 5-Fluorouracil-based chemotherapeutic regimen for treating a tumor in a patient comprising:

(a) obtaining a tumor sample from the tumor;

(b) isolating mRNA from a tumor sample;

(c) subjecting the mRNA to amplification using a pair of oligonucleotide primers having of the sequence of SEQ ID: 7[,or which is substantially identical thereto] or an oligonucleotide primer at least 80% identical therewith and hybridizes to a complement of SEQ ID NO: 7 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of Exon 6 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 8, and an oligonucleotide having the sequence SEQ ID: 8[, or which is substantially identical thereto] or an oligonucleotide primer at least 80% identical therewith and

hybridizes to a complement of SEQ ID NO: 8 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of Exon 6 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 7;

to obtain an amplified sample;

(d) determining the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample;

(e) comparing the amount of *Dihydropyrimidine Dehydrogenase* (*DPD*) mRNA in the amplified sample with a predetermined threshold level for *DPD* expression;

(f) determining a 5-Fluorouracil-based chemotherapeutic regimen for said patient based on the difference in amount of *DPD* mRNA in the amplified sample and the threshold level for *DPD* gene expression.

24. (Amended) The method of claim 23, wherein said predetermined threshold level of *DPD* gene expression is about 2.0 to about 2.5 times that of an internal control gene expression level.

25. The method of claim 23, or 24, wherein said internal control gene is β-actin.